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(54) Preparation and magnetic properties of very small magnetite-dextran particles.

(57) Particles comprising a magnetically responsive material, preferably magnetite, and a dispersant, preferably dextran, wherein at least 90% of said particles have a particle size range of about 2 to about 10 nm are described, together with a method for their preparation, comprising admixing a magnetically responsive material with a dispersant to form an admixture, conveniently suspended in a gel phase, and contacting said first admixture with a base, such as ammonium hydroxide, for a time period and under conditions appropriate to form a magnetically responsive material-dispersant particle.

The use of the particles for a method of diagnosis comprising administering to a mammal a contrast effective amount of these particles suspended or dispersed in a physiologically tolerable carrier with generation of an NMR image of the mammal is also described.

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FIELD OF THE INVENTION

The present invention relates to a diagnostic agent containing particles having a magnetic moment, which diagnostic agent is useful in nuclear magnetic resonance (NMR) imaging, and a method for making that diagnostic agent. The invention further relates to the use of such diagnostic reagents in pharmaceutical compositions.

BACKGROUND OF THE INVENTION

NMR contrast agents in commercial use at the present time are paramagnetic. They consist of transition metals (such as manganese), elements of the lanthanide series (such as gadolinium), or stable organic free radicals. More recently, particles possessing magnetic moments have attracted a great deal of attention because they are effective at concentrations less than one millionth of the effective concentrations of non-particulate materials. Renshaw *et al.*, Magnetic Resonance Imaging 4:351-357 (1986).

In addition, these non-particulate agents affect both the spin lattice (T_1) and the spin-spin (T_2) relaxation time of protons in different tissues whereas the particulate materials such as the magnetic iron oxides primarily reduce the T_2 relaxation time. Josephson *et al.*, Magnetic Resonance Imaging 6:647-653 (1988).

Several reports relate enhanced T_2 relaxation rates of normal tissue but not of tumor tissues. By using the T_2 weighted image sequence, good contrast can be obtained between the tumor and the surrounding healthy tissue at very low levels of particulate contrast agent see, for example, Magin *et al.*, Magnetic Imaging in Medicine 20:1-16 (1991); Weissleder *et al.*, Radiology 162:494-498 (1990); Saini *et al.*, Radiology 167:211-216 (1987); *Id.* 217-222; Hahn *et al.*, Radiology 164:37-41 (1987).

The size of the magnetic particulates plays a very important role in the selectivity of absorption of contrast agent by tissue. When the diameter of the magnetic particles is in the range of 0.03 to 1 micron (or larger), they are rapidly cleared from the blood by the mononuclear phagocytic system (MPS) of the spleen and liver and no particles are found in other tissues (Weissleder *et al.*, Radiology 175:489-493 (1990)). As the sizes of the particles decrease to the 0.01 micron (10 nanometer, nm) range and lower, the particles are not absorbed by the MPS of the liver and spleen and have a longer life-time in the circulatory system and thus have uptake by lymph nodes, bone marrow, and other organs.

Current methods of producing magnetic particles produce a reaction product which possesses a wide range of magnetic particle sizes. The so-called ultrasmall sizes are obtained by fractionation of the reaction mixture. This involves high speed centrifugation, and the use of gel chromatographic columns as in U. S. Patent No. 5,055,288. Such fractionation results in very low yields of the desired product.

Molday *et al.*, J. Immun. Meth. 52:353-367 (1982), describe the preparation of particles in the size range of 30-40nm which have an electron dense core of 15nm. Molday uses centrifugation and gel filtration chromatography to fractionate and purify the resulting particles, similar to the methods of U.S. Patent No. 5,055,288. Other techniques for the preparation of small magnetite particles for magnetic resonance imaging (MRI) have followed Molday in the use of dextran, with the exception of a liposome based system as described in U.S. Patent No. 5,088,499.

U.S. Patent No. 4,965,007 describes magnetite encapsulated by a gelatin-gum arabic coacervate for magnetic separations. That material is not suitable for MRI by virtue of the physiological limitation imposed by the encapsulating system.

U.S. Patent No. 4,985,233 describes a method of diagnosis by the administration of a diagnostic agent comprising a paramagnetic compound carried by a particulate macromolecular product. In one embodiment, carboxymethyl gel beads are swelled in the presence of $\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$ and dried and swelled again in the presence of NaOH. The resulting particles have sizes ranging from 40 to 160 microns.

The specification of WO Patent 8903-675-A (Carbomatrix AB) describes a method for preparing superparamagnetic particles by combining a carbohydrate polymer, ferric chloride, ferrous chloride and NaOH. The resulting particles are said to have a size range of 0.35 to 1.22 microns.

All of the literature methods for the preparation of magnetite (Fe_2O_3) for use as a magnetic resonance contrast agent produce a wide range of magnetic particle sizes. For MRI, the magnetic crystallite size preferably is less than about 10 nm and these are obtained by fractionation of the reaction mixture using high speed centrifugation and gel chromatography.

The present invention describes a new method for the synthesis of particles, which is capable of high yields, comprising a magnetically responsive material and a dispersant in which a very narrow size distribution is obtained without the necessity for fractionation procedures.

SUMMARY OF THE INVENTION

Thus according to the present invention there are provided particles comprising a magnetically responsive material and a dispersant, wherein at least 90% of said particles have a particle size between about 2 and about 10 nm. Preferably, the particle size range is from about 2 to about 5 nm. These particles are particularly useful as diagnostic agents in NMR imaging.

The present invention is also directed to a method for the preparation of such particles comprising admixing a magnetically responsive material with a dispersant to form an admixture, and contacting said first admixture with a base for a time period and under conditions appropriate to form a magnetically responsive material-dispersant particle. In a preferred embodiment, the magnetically responsive material is magnetite, the dispersant is dextran, and the base is ammonium hydroxide. In a further preferred embodiment, the admixture is suspended in a gel phase. A preferred gel phase is formed by gelatin or by polyvinyl alcohol. Contacting may take place in a gas, liquid, or gel phase.

The present invention is further directed to a method of diagnosis comprising administering to a mammal a contrast effective amount of these particles suspended or dispersed in a physiologically tolerable carrier and generating an NMR image of said mammal.

DETAILED DESCRIPTION OF THE INVENTION

This invention is directed to particles of particle size range from about 2 to about 10 nm, comprising a magnetically responsive material and a dispersant.

It is also directed to a method by which a base, such as ammonium hydroxide, is added to an admixture of magnetically responsive material, such as an iron salt, and a dispersant, such as dextran, to obtain particles with the required diameter range. It is necessary to add the hydroxide so that the local hydroxide concentration in the reaction zone is not in excess as the iron oxide product is formed. With alkali metal hydroxides, this may be accomplished by suspending the iron salt-dextran mixture into a gel phase and allowing the hydroxide to diffuse into the gel. In the case of volatile hydroxides, such as ammonium hydroxide, the addition may be made through the gas phase, which is in contact with a solution of the iron salt-dextran.

As used herein, the phrase "particle size" refers to a mean particle size as measured by conventional particle size measuring techniques well known to those skilled in the art, such as sedimentation field flow fractionation, photon correlation spectroscopy, or disk centrifugation. The phrase "particle size range of about 2 to about 10 nm" as used herein means that at least 90% of the particles have a particle size of between about 2 nm and about 10 nm when measured by the above-noted techniques. It is preferred that at least 95% and, more preferably at least 99% of the particles have a particle size range from about 2 nm to about 10 nm.

The amount of magnetically responsive material present in the compositions of the present invention depends upon numerous factors, including stability, temperature, pH of the reaction, and the like. A preferred concentration (weight/volume) of magnetically responsive material is from about 0.5 to about 20%, more preferably 1 to 8%.

The magnetically responsive material may be a metal oxide of iron, nickel or cobalt. A magnetic particle particularly useful in the present invention is magnetite (Fe_3O_4). This material is prepared by adding alkali to a mixture of ferrous and ferric salts.

The preparation of magnetite is described by equation (1)



The base, which serves as the hydroxide source (OH^-), may be either inorganic (for example, sodium, potassium, or ammonium hydroxide) or organic (tetra-alkyl ammonium hydroxide).

A dispersing agent (dispersant) is typically present during the reaction. A preferred dispersing agent is dextran. Examples of other dispersing agents include water soluble polysaccharides such as glucans, e.g. starch, amylose, amylopectin (including macromolecular dextrans thereof), glycogen, dextran and pullulan, fructans, e.g. inulin and levan, and other physiologically tolerable polysaccharides of vegetable, microbial or animal origin. Another example is the so-called polyglucose obtained by polymerization of glucose. Other examples include macromolecular products obtained by cross-linking carbohydrates or sugar alcohols (e.g. mannitol and sorbitol) with at least one bifunctional cross-linking agent, e.g. with epichlorohydrin or diepoxides or corresponding halogen hydrins. An example of such a product is Ficoll (Pharmacia Fine Chemicals AB, Uppsala, Sweden) which is obtained by cross-linking sucrose with the aid of epichlorohydrin.

(see e.g. SE-B-209 018 and U.S. Patent No. 3,300,474).

The optimal amount of the dispersant can depend, for example, upon the particular magnetically responsive material selected, the critical micelle concentration of the dispersant if it forms micelles, the hydrophilic/lipophilic balance (HLB) of the stabilizer, the melting point of the stabilizer, its water solubility, the surface tension of water solutions of the stabilizer, etc. The dispersant preferably is present in an amount of about 0.1-10 mg/m² surface area of the therapeutic or diagnostic agent. The dispersant can be present in an amount of 0.1-90%, preferably 1-75%, more preferably 10-60%, and most preferably 10-30% by weight based on the total weight of the dry particle.

Dextran was first described for use in oral iron supplements as in Ricketts et al., *Nature* 208:237 (1965). The first mention of its use in stabilizing magnetite particles for use in magnetic resonance imaging appeared in 1978 in Ohgushi et al., *J. Mag. Reson.* 29:599-601 (1978).

In practice, the hydroxide is added to the iron salt/dextran mixture. Kronick et al., *J. Biochem. Biophys. Meth.* 12:73-80 (1986) reverse this addition, such that the iron salt-dextran solution is added to an excess of alkali. U.S. Patent No. 5,102,652 discloses a method where the iron salt solution is added to the alkali-dextran.

The amount of base to be added depends upon a number of factors, including pH of the solution, the nature of the dispersant, the nature of the magnetically reactive material, and the like. A preferred amount of base is an amount sufficient to raise the pH of the suspension to between about 8 and 11.

In one embodiment, the magnetically reactive material-dispersant admixture is suspended in a gel phase. A gel phase, as used herein, is a colloidal suspension of a liquid in a semisolid, that forms a jellylike material. Examples of gelling agents useful in forming a gel phase include porcine skin gelatin, bovine skin gelatin, plant gelatin (such as carageenan), agarose, polyacrylamide, and polyvinyl alcohol. Preferred gelling agents include porcine skin gelatin and polyvinyl alcohol.

The amount of gelling agent useful in the processes of the present invention depends upon numerous factors, including the pH of the solution, the dispersant used, the magnetically reactive material used, the nature of the contacting with the gelled phase, and the like. In a preferred embodiment, the concentration (weight/volume) of gelling agent is from about 1 to about 10%.

Contacting the magnetically reactive material-dispersant admixture in the liquid phase with the base can be by liquid/liquid or gas/liquid interaction. Where the contacting is by liquid/liquid interaction, the magnetically reactive material-dispersant admixture is contacted with the base by admixing. Where the contacting is by gas/liquid interaction, the magnetically reactive material-dispersant admixture is contacted with the gas phase of a volatile base such as ammonium hydroxide or a volatile amine such as methyl or ethylamine.

Contacting the magnetically reactive material-dispersant admixture suspended in the gel phase with the base can be by liquid/gel or gas/gel interaction. Where the contacting is by liquid/gel interaction, the gelled magnetically reactive material-dispersant admixture is overlaid with the base. Where the contacting is by gas/gel interaction, the gelled magnetically reactive material-dispersant admixture is contacted with the gas phase of a volatile base such as ammonium hydroxide or a volatile amine such as methyl or ethylamine.

In a method of diagnosis comprising administration of the particles to a mammal, a "contrast effective amount" of particles is that amount necessary to provide tissue visualization with magnetic resonance imaging. Means for determining a contrast effective amount in a particular subject will depend, as is well known in the art, on the nature of the magnetically reactive material used, the mass of the subject being imaged, the sensitivity of the magnetic resonance imaging system and the like.

After administration of these particles, the subject mammal is maintained for a time period sufficient for the administered particles to be distributed throughout the subject and enter the tissues of the mammal. Typically, a sufficient time period is from about 20 minutes to about 90 minutes, preferably from about 20 minutes to about 60 minutes.

The particles are visualized by imaging that tissue with a magnetic resonance imaging system. The visualization of the particles can be accomplished with commercially available magnetic imaging systems such as a General Electric 1.5 T Signa imaging system [¹H resonant frequency 63.9 megahertz (MHz)]. Commercially available magnetic resonance imaging systems are typically characterized by the magnetic field strength used, with a field strength of 2.0 Tesla as the current maximum and 0.2 Tesla as the current minimum.

For a given field strength, each detected nucleus has a characteristic frequency. For example, at a field strength of 1.0 Tesla, the resonance frequency for hydrogen is 42.57 MHz; for phosphorus-31 it is 17.24 MHz; and for sodium-23 it is 11.26 MHz.

A carrier or diluent is a material useful for administering the particles and must be "pharmacologically acceptable" in the sense of being compatible with the other ingredients of the composition and not

deleterious to the recipient thereof. Thus, as used herein, the phrases "physiologically tolerable" and "pharmacologically acceptable" are used interchangeably and refer to molecular entities and compositions that do not produce an allergic or similar untoward reaction, such as gastric upset, dizziness and the like, when administered to a mammal. The physiologically tolerable carrier can take a wide variety of forms depending upon the preparation desired for administration and the intended route of administration.

As an example of a useful composition, the particles of the present invention can be utilized in liquid compositions such as sterile suspensions or solutions, or as isotonic preparations containing suitable preservatives. Particularly well-suited for the purposes of the present invention are injectable media constituted by aqueous injectable isotonic and sterile saline or glucose solutions.

The agents can also be administered in the form of liposomes. As is known in the art, liposomes are generally derived from phospholipids or other lipid substances. Liposomes are formed by mono- or multilamellar hydrated liquid crystals that are dispersed in an aqueous medium. Any non-toxic, physiologically acceptable and metabolizable lipid capable of forming liposomes can be used. The present compositions in liposome form can contain stabilizers, preservatives, excipients, and the like in addition to the agent.

The preferred lipids are phospholipids and phosphatidyl cholines (lecithins), both natural and synthetic.

Methods of forming liposomes are known in the art, see for example, Prescott, Ed., Methods in Cell Biology, Volume XIV, Academic Press, New York, N.Y. (1976), p. 33 *et seq.*

The invention will now be illustrated with reference to the following examples but is in no way to be construed as limited thereto.

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Example 1.

Magnetite-dextran particles were produced according to the methods of U.S. Patent Nos. 4,770,183 and 4,827,945 (AMI) and Josephson *et al.*, *op. cit.* These methods utilize a strong base that is titrated into a reaction mixture consisting of a mixture of iron salts and dextran. After the reaction is completed, the product is centrifuged and fractionated in a gel column. The present example uses sodium hydroxide. A similar synthesis utilizing ammonium hydroxide in place of sodium hydroxide gave similar results, although the amount of magnetite separated by the centrifuge was less.

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(i) 3.8 g $\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$, 1.4 g $\text{FeCl}_2 \cdot 4\text{H}_2\text{O}$ (both purchased from Aldrich Chemical Company) and 10 g dextran (Pharmacia Fine Chemicals) were dissolved in 75 ml water with stirring via a magnetic stirring bar.

(ii) 80 ml 1N NaOH was added over a 30 minute time period, with vigorous stirring. As the base was added, the solution changed in color from black to brown and then green. The final pH value of the solution was approximately 11.5.

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(iii) The solution from step (ii) was then heated with stirring to 80°C at which point the solution was dark brown. At this point, the pH of the solution was rapidly lowered to a value of 7 using 5N HCl with rapid cooling to below 10°C. There was an appreciable amount of black magnetite on the magnetic stirring bar.

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(iv) The suspension from step (iii) was then centrifuged for 1 hour at 5°C and 13,500 rpm in a DuPont Sorvall RC-5B refrigerated superspeed centrifuge. An appreciable amount of black magnetite was collected at the bottom of the centrifuge tube.

(v) The supernatant liquid collected from the tubes was passed through a 0.2 micron Nalgene filter. The filter was gray, indicating that the centrifuge allowed a small fraction having a particle diameter greater than 0.2 micron to remain in suspension.

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(vi) A 20 ml sample of the above was fractionated in a column (5 cm diameter, 40 cm length) packed with Sepharose, CL4B (Pharmacia), using a Tris buffer (3.075 g Tris base, 6.25 g NaCl, 24 ml 1N HCl diluted to 1 l) as the eluant. The flow rate was 4 ml/minute and a brown fraction was left at the upper part of the column. The total volume obtained in the fractionation collector was 90 ml.

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(vii) The middle 50 ml of the product from step (vi) was dialyzed in the same Tris buffer using a dialysis membrane with a molecular weigh cutoff of 6000-8000 daltons. Dialysis was carried out at 5°C.

(viii) After 24 hours, the material was removed from the dialysis bag and concentrated in an Amicon diafiltration cell using a YM10 Diaflo filter (molecular weight cutoff of 10,000 daltons) and 170 kPa (25 psi) of argon gas. The purified suspension was stored-at 5°C and was now ready for particle size and magnetic characterization.

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Example 2.

In this example, magnetite particles were prepared according to the Franklin Institute method [Kronick et al., J. Biochem. Biophys. Meth. 12:73-80 (1986)]. This method utilizes the same ingredients as described in Example 1 but with a reverse order of mixing. Here, the iron salt-dextran solution mixture was added rapidly to a hot solution of sodium hydroxide.

- (i) A solution of $\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$ (0.18 g), $\text{FeCl}_2 \cdot 4\text{H}_2\text{O}$ (0.14g) and dextran T-10 (4g), all in 60 ml water was added to a hot (80°C) solution of sodium hydroxide (6.2g NaOH in 60 ml water) very rapidly while stirring with a magnetic stir bar.
- (ii) The solution from step (i) was cooled rapidly to 5°C and 13 ml of 12N HCl added so that the final pH of the solution had a value of approximately 7.
- (iii) The suspension from step (ii) was centrifuged as in step (iv) of Example 1. A considerable amount of black magnetite separated and the supernatant liquid was passed through a 0.2 micron filter which turned gray indicating particles in the suspension larger than 0.2 micron.
- (iv) The suspension was fractionated in a gel column and using the steps (vi through viii) as in Example 1. The suspension was stored at 5°C .

Example 3.

A solution consisting of 5.42% $\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$ and 2.00% $\text{FeCl}_2 \cdot 4\text{H}_2\text{O}$ was prepared. This composition has a mole ratio of ferric ion:ferrous ion of 2:1, which is the stoichiometric ratio for the formation of magnetite. Also, premixing the two salts confers stability on the mixture. Ferrous solutions form insoluble precipitates on standing. 7.01 ml of the above solution was mixed with 50 ml 20% dextran (Pharmacia, T10) and the mixture was passed through a 0.2 micron filter to remove any insoluble impurities.

A 150 ml beaker of this mixture was placed into a 600 ml beaker, and 60 ml concentrated ammonium hydroxide was placed in the space between the two beakers. The system was closed off from the atmosphere and stirred with a magnetic stirrer. After 1 hour, the solution had turned a dark burgundy color, and the pH value of the solution was 10.4. The pH of the solution was lowered to a value of 7.0 with concentrated HCl.

There was no sign of magnetite adhering to the stirring bar and filtering through 0.1 micron Nalgene (nylon) filter did not produce any residue, in contrast with Examples 1 and 2.

Excess dextran was removed by diafiltration (Amicon, PM30) using argon gas at 170 kPa (25 psi). Four filtrations were carried out by the process of diluting the residue in the Amicon cell with water and diafiltering to the point where the dextran level in the filtrate was below 0.2%.

Since virtually no iron was found in the filtrates (using Atomic Absorption analysis), the reaction gave virtually 100% yield.

Example 4.

This Example provides data summarizing the size and magnetic resonance data obtained with the particles prepared in Examples 1-3, above.

Table 1 compares the particle sizes, crystallite dimensions, and Magnetic Resonance Relaxivities of the magnetite-dextran particles prepared according to the above examples. The sizes determined by lattice fringe imaging are the fundamental crystallite sizes independent of the amount of absorbed dextran. The methods of the present invention, embodied in Example 3, yield populations with the smallest diameters and with very narrow size distributions. The diameters determined by Laser Light Scattering includes the thickness of the dextran shell and they are all approximately the same. However, the diameters of the particles produced in the present invention show a narrow size distribution. The Transmission Electron Micrographs for particles prepared by the methods of Examples 1 and 2 were difficult to interpret because of severe particle clumping. However, the particles of the present invention, while showing aggregation, were still very well dispersed.

TABLE 1.

	<u>PARTICLE SIZE</u>		<u>CRYSTALLITE SIZE</u>		<u>RELAXIVITY</u>
	<u>Ex. TEM¹</u>	<u>LASER LS²</u>	<u>Lattice Fringe Imaging</u>		<u>R₂/R₁</u>
	d (nm)	σ (nm)	d (nm)	σ	
10	1.	5-30	32	17.4	8.1 2.4 4 (Lit.) ³
15	2.	5-20	36	12	4.6 0.6 3.5
20	3	2-10	34	1.6	4.1 0.25 5.5
25	MR MSRTS: 40°C, 0.47 Tesla (20 MHZ for Protons).				
	¹ TEM = Transmission Electron Microscope.				
	² LASER LS = LASER Light Scattering.				
	³ Josephson <i>et al.</i> , op. cit., 651.				

The ratio of the magnetic resonance relaxivities (R_2/R_1) is an indication as to the value of the compound as a negative imaging agent. The composition of the present invention has a large R_2 value, indicating that this composition is an excellent negative imaging agent. A future commercial material (AMI-25, Advanced Magnetics Inc.) has a ratio of 4 for a mean particle size of 72 nm.

Example 5.

This Example illustrates the use of a gel matrix containing the iron salts in suspension and which allows the use a strong caustic base solution instead of ammonia vapor.

The following was mixed into a 10% solution of gelatin derived from pigskin (with an isoelectric point of 8.5). The iron salts were dissolved at a temperature of 45°C at which the gelatin is liquid.

To 10 ml gelatin solution was added 0.076g ferric chloride•6H₂O and 0.028g ferrous chloride•4H₂O. The solution was placed into a test tube and cooled to room temperature at which point it formed a rigid gel. It was overlayed with 10 ml 10% NaOH solution and placed into a refrigerator (at 4°C) overnight.

18 hours later, the first 12mm of gelatin had turned to a characteristic red-brown color typical of small magnetite particles. The excess alkali was washed off the surface of the solidified gelatin, and the temperature of the gelatin was raised to 45°C to melt the gelatin. The liquid gelatin was diluted to 1% gelatin so that it would remain liquid at room temperature, and filtered through a 0.2 filter. No residue was retained by the filter.

Example 6.

This example is similar to Example 5 except that polyvinyl alcohol (PVA; DuPont Elvanol 71-30) was used instead of gelatin.

A small amount of boric acid was added to the iron salt-PVA mixture. This causes the PVA to gel when alkali is added. After overlaying with 10% sodium hydroxide and storing overnight at room temperature, the presence of magnetite, as evidenced by the characteristic red-brown color, could be detected in the upper 1 cm of the solidified gel. This gel system was liquified by mixing the solidified gel with dilute acetic acid and tested for the presence of large particles by filtering through a 0.2 filter. None was detected.

Claims

1. Particles comprising a magnetically responsive material and a dispersant, wherein at least 90% of said particles have a particle size between about 2 and about 10 nm.
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2. Particles as claimed in claim 1 wherein said magnetically responsive material is magnetite.
3. Particles as claimed in claim 1 wherein said dispersant is dextran.
- 10 4. Particles as claimed in claim 1 wherein at least 90% of said particles have a particle size between about 2 and about 5 nm.
5. A method for the preparation of the particles as claimed in any one of the preceding claims comprising admixing a magnetically responsive material with a dispersant to form an admixture, and contacting
15 said admixture with a base for a time period and under conditions appropriate to form a magnetically responsive material-dispersant particle.
6. A method as claimed in claim 5 wherein said first admixture is suspended in a gel phase.
- 20 7. A method as claimed in claim 6 wherein said gel phase is formed by gelatin.
8. A method as claimed in claim 6 wherein said gel phase is formed by polyvinyl alcohol.
- 25 9. A method of diagnosis comprising administering to a mammal a contrast effective amount of the particles as claimed in any one of claims 1 to 4 suspended or dispersed in a physiologically tolerable carrier and generating an NMR image of said mammal.

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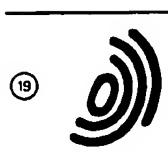
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PARTIAL EUROPEAN SEARCH REPORT

Application Number

which under Rule 45 of the European Patent Convention EP 93 20 3162
shall be considered, for the purposes of subsequent
proceedings, as the European search report

DOCUMENTS CONSIDERED TO BE RELEVANT			
Category	Citation of document with indication, where appropriate, of relevant passages	Relevant to claim	CLASSIFICATION OF THE APPLICATION (Int.CLS)
D, X	RADIOLOGY, vol. 175, no. 2, 1990 pages 489-493, R. WEISSLEDER ET AL. 'ULTRASMALL SUPERPARAMAGNETIC IRON OXIDE: CHARACTERIZATION OF A NEW CLASS OF CONTRAST AGENTS FOR MR IMAGING' * the whole document * ---	1-4, 9	A61K49/00
D, X	JOURNAL OF BIOCHEMICAL AND BIOPHYSICAL METHODS, vol. 12, no. 1/2, 1986 pages 73-80, P. KRONICK ET AL. 'USE OF SUPERPARAMAGNETIC PARTICLES FOR ISOLATION OF CELLS' * the whole document *	1-5	
Y	---	9	
		-/-	
			TECHNICAL FIELDS SEARCHED (Int.Cl.S)
			A61K
INCOMPLETE SEARCH			
<p>The Search Division considers that the present European patent application does not comply with the provisions of the European Patent Convention to such an extent that it is not possible to carry out a meaningful search into the state of the art on the basis of some of the claims</p> <p>Claims searched completely :</p> <p>Claims searched incompletely :</p> <p>Claims not searched :</p> <p>Reason for the limitation of the search:</p>			
<p>see sheet C</p>			
Place of search	Date of completion of the search	Examiner	
THE HAGUE	27 July 1995	Hoff, P	
CATEGORY OF CITED DOCUMENTS		T : theory or principle underlying the invention E : earlier patent document, but published on, or after the filing date D : document cited in the application L : document cited for other reasons ----- & : member of the same patent family, corresponding document	
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Application Number

EP 93 20 3162

Category	Citation of document with indication, where appropriate, of relevant passages	Relevant to claim	CLASSIFICATION OF THE APPLICATION (Int.CI)
			TECHNICAL FIELDS SEARCHED (Int.CI)
X	INTERNATIONAL JOURNAL OF PHARMACEUTICS, vol. 40, no. 3, 1987 pages 201-206, M.I. PAPISOV ET AL. 'MAGNETIC DRUG TARGETING.I.' * abstract * * page 202 * * page 206 * ---	1-4	
Y	WO-A-90 01899 (ADVANCED MAGNETICS, INCORPORATED) 8 March 1990 * abstract * * page 13, line 11 - line 24 * * page 17, line 10 - page 19, line 3; claims; table I * ---	9	
Y	MAGNETIC RESONANCE IN MEDICINE, vol. 22, no. 2, 1991 pages 435-442, A. IANONNE ET AL. 'BLOOD CLEARANCE OF DEXTRAN MAGNETITE PARTICLES DETERMINED BY A NONINVASIVE IN VIVO ESR METHOD' * the whole document * ---	9	
D,A	WO-A-88 00060 (ADVANCED MAGNETICS INCORPORATED) 14 January 1988 * abstract * * page 12, line 15 - page 14, line 19 * * page 21, line 5 - line 34 * * page 42, line 19 - line 32 * * page 44, line 5 - page 45, line 19; claims 2-25; table II * ---	1-9	
		-/-	



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DOCUMENTS CONSIDERED TO BE RELEVANT			CLASSIFICATION OF THE APPLICATION (Int.Cl.5)
Category	Citation of document with indication, where appropriate, of relevant passages	Relevant to claim	TECHNICAL FIELDS SEARCHED (Int.Cl.9)
P,X	JMR, vol. 3, July 1993 pages 641-648, J.W.M. BULTE ET AL. 'FREQUENCY DEPENDENCE OF MR RELAXATION TIMES.II. IRON OXIDES' * the whole document *	1-4, 9	



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Remark: Although claim 9
is directed to a method of
treatment of the human/animal
body (Art. 52(4) EPC) the search
has been carried out and based on
the alleged effects of the
compound/composition